A PECULIAR NUCLEAR ELEMENT IN THE MALE REPRODUCTIVE CELLS OF INSECTS.

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In working out the spermatogenesis of one of the Locustidae, *Xiphidium fasciatum*, a nuclear element not heretofore described arrested the attention of the writer, and will here be given preliminary notice in order that other investigators in the same line of work may be induced to look for it in their preparations. For the sake of convenience this structure will be called, provisionally, an accessory chromosome.

While but the one species has been studied and the appearance of the new element noted, a study of figures representing insect spermatogenesis indicates that it is not merely a specific character. Such an inspection of the figures given by different writers shows clearly that the body under discussion has been observed, but that its true character has not been recognized. The reason for this may be apparent later, when the changes it undergoes have been described. The truly remarkable and striking nature of the element and its obvious importance in the formation of the spermatozoön render a more thorough knowledge of it highly desirable.

In order to make clear the true character of the body, an account of its behavior in different stages of the maturation of the spermatozoön will be given, and then attention will be called to the most striking features distinguishing it.

As it first appears in the spermatogonia of *Xiphidium fasciatum*, there would be no hesitation in calling it a nucleolus except for its unusual situation on the surface of the nuclear vesicle. It is a small, irregularly rounded body, and lies immediately under the nuclear membrane (Fig. 1). Before the division figure is established, however, it takes on the form of a thread which becomes "U"-shaped (Fig. 2). Still further contraction ensues, and by the time of the metaphase the

thread has become very short and thick and is bent in the middle with an obtuse angle so as to resemble a boomerang. At this time, it may be observed lying at one side of the circle of chromosomes arranged in the equatorial plate, and plainly

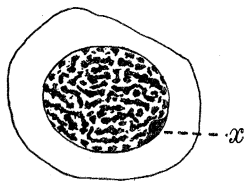

FIG. 1. — Prophase of spermatogonia showing the accessory chromosome, marked "x," applied to the surface of the nuclear vesicle.

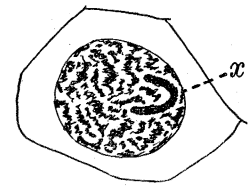

FIG. 2. — Later prophase of the spermatogonial stage in which the accessory chromosome has become "U"-shaped.

distinguishable from them by reason of its greater length (Fig. 3). From the pole the chromatin appears as a broad, fenestrated plate, and the accessory chromosome is indistinguishable from the ordinary ones (Fig. 4).[1] Because of the rapidity of the division none of the anaphases are to be seen, but in the telophases the ordinary chromosomes of the cell may be seen

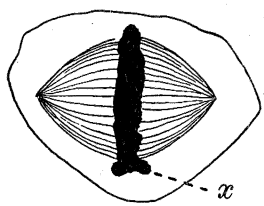

FIG. 3. — Metaphase of the spermatogonia showing the accessory chromosome applied to the periphery of the circle of chromosomes.

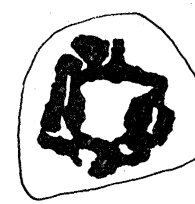

FIG. 4. — The same stage as represented in Fig. 3, but viewed from the pole.

grouped in the typical manner at the two ends of the spindle, while extending down towards the equatorial plate from each

[1] This peculiar arrangement of the chromatic mass is very striking, and is clearly due to a strong concentration of the nuclear elements. When the broad plate is cut squarely across, near one surface, the ends of the chromosomes may be observed as isolated bodies, but near the center of the group they lose their individuality in the mass. It is possible that the effect is due to improper fixation, but since all the cells around the follicle containing the spermatogonia, and even the cytoplasm of the spermatogonia themselves, is excellently preserved, this seems hardly probable.

mass is a half of the boomerang-shaped body which has been divided longitudinally in the same manner as the ordinary chromosomes. When this is observed projecting down from the middle of the group of chromosomes, thus presenting its broadest surface, it exhibits the form of an attenuated "U," the curved end of which is in contact with that of its fellow on the

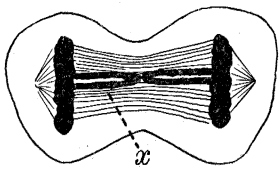

FIG. 5. — Late anaphase of the spermatogonia exhibiting the accessory chromosomes divided and still attached at their ends in the equatorial plate.

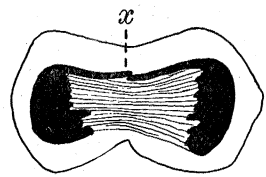

FIG. 6. — The same viewed from the side.

other side (Fig. 5). In cases where the section is cut so that the thread depends from the side of the group, the two chromatic masses present, roughly, the appearance of two hands with the index fingers pointing toward each other (Fig. 6).

In the resting stage of the spermatocyte that succeeds the appearance just described, the accessory chromosome again appears as it did in the resting stage of the spermatogonia, and

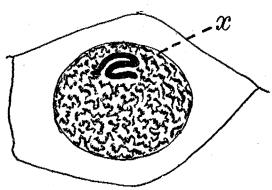

FIG. 7. — Early prophase of the spermatocyte. The accessory chromosome in the form of a coiled thread.

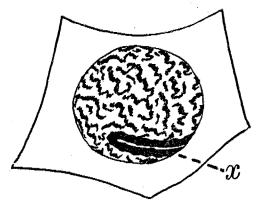

FIG. 8. — Later prophase of the spermatocyte. The accessory chromosome "U"-shaped.

would easily be taken for an ordinary nucleolus. Soon, however, it commences to assume a threadlike form which finally results in the production of a long "U"-shaped body, a form that is retained during the greater part of the spireme stage (Figs. 7–9). In this condition, it lies at the surface of the vesicle and stains in its usual intense manner. Concurrently with the formation of the "rings" from the spireme thread, it

commences to shorten and grows into the form of a horseshoe, and is finally to be distinguished from the chromatic rings only by its deeper staining quality and by the smoothness of its outline (Fig. 10). In the formation of the mitotic figure of the first spermatocyte division, it assumes its position on the outside of the group of chromosomes as it did in the spermatogonial division, and again has the boomerang shape that marked its appearance in the early figures (Fig. 11). When the chromatin

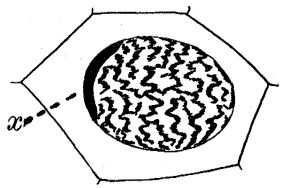

FIG. 9. — A somewhat later spermatocyte prophase. The accessory chromosome viewed from the side.

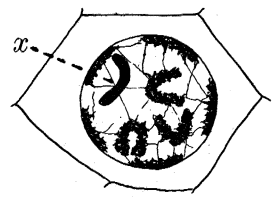

FIG. 10. — "Ring" stage of the spermatocyte. The accessory chromosome distinguishable from the remaining nuclear elements by reason of its greater density and smoother outline.

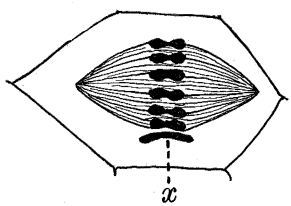

FIG. 11. — Metaphase of the spermatocyte. The accessory chromosome in the shape of a boomerang at one side of the group of chromosomes.

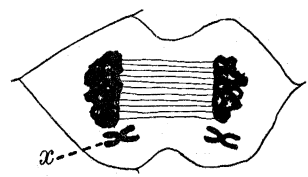

FIG. 12. — Late anaphase of the spermatocyte. The accessory chromosomes in the form of double horseshoes in the two daughter-cells.

separates and moves to the two poles, the accessory chromosome divides longitudinally and presents the appearance of two horseshoes with their rounded ends in contact (Fig. 12). In the second spermatocyte division, apparently the same process is followed.

The recently formed spermatids possess a nucleus in which the ordinary chromatin is extremely scant (Fig. 13) and very weak in staining power, while the accessory chromosome shows as prominently as ever and stains in the same uniform manner. It is not easy to trace out the part that the different elements

of the nucleus take in the formation of the spermatozoön, but in the light of present knowledge it appears as if the accessory chromosome was prominently concerned in the formation of the head. The nucleolus-like body that results from the last spermatocyte division, which has again taken up its position on the surface of the nucleus, becomes vacuolated and forms a covering for the nuclear vesicle. Gradually this collects at the end of the pear-shaped vesicle, and by the usual process of condensation and arrangement of the chromatic and achromatic parts of the cell the spermatozoön is formed.

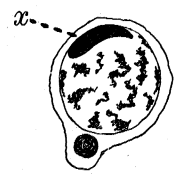

FIG. 13. — Spermatid showing the strongly staining accessory chromosome and the weakly staining chromatin.

It being the purpose of the present article merely to call attention to the changes taking place in the accessory chromosome, no attention will be paid to the part played by the other cell structures, except as they have some bearing upon the behavior of this body.

In seeking to point out the features that characterize this peculiar nuclear element, perhaps the most striking thing to be noticed is the almost uniform staining power exhibited. While the ordinary chromatin gradually and progressively weakens in staining ability, the accessory chromosome retains its original affinity for the haematoxylin and basic anilines undiminished. As a consequence of this, in all the cells of the testes, the accessory chromosome is at once distinguishable. Only in the early spermatogonia, when the chromatin stains most strongly, is there any difficulty in observing this peculiar element. As the cells progress toward the formation of the spermatozoön, a greater and greater difference arises between the chromatin and its companion element, until in the spermatid so preponderant has become the volume of the accessory chromosome that one is almost irresistibly driven to the conclusion that it is chief in importance.

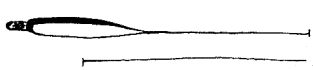

FIG. 14. — Almost mature spermatozoön.

This variation in staining capacity of the chromatin and nucleolus (?) has not escaped the observation of other investigators. In describing the staining reaction of the different

elements of the testicular cells of *Caloptenus femur rubrum*, Wilcox[1] notes that, while ordinarily the chromatin and the nucleolus stain red and the cytoplasm green, by double staining in safranin and victoria green, yet "in *some*[2] stages the chromosomes were stained green, indicating that a chemical change takes place in the chromatic substance. But even in such cases the nucleolus was bright red."

Again, he states that "by this method (Henneguy's) the chromosomes and nucleoli are stained bright red." With reference to the changes occurring in *Cicada*, this alternation of staining power is noted particularly in the following language: "By the safranin and victoria green method the chromosomes stain red, though not so deeply as the nucleoli. At later stages the chromosomes assume a green color while the nucleoli continue to stain red. In still later stages the chromosomes again take the red."

All these color reactions ascribed to the nucleolus by Wilcox are strictly parallel to those exhibited by the accessory chromosome in *Xiphidium* preparations, and there can be little doubt that the two elements are identical. Added force is lent to this view by the appearance of the cells shown in Fig. 108 by Wilcox, in which the nucleolus is represented just as the accessory chromosome appears in the sperm-forming cells of *Xiphidium*. Moreover, with regard to the spermatogonial divisions, he says: "In most cases a nucleolus is to be seen during the prophases. In Fig. 106 there is in the nucleus a body (nucleolus (?)) which seems to have recently divided."

Later Wilcox refers to this same body, apparently, as a centrosome which becomes included in the nuclear membrane and goes to form the "neck" of the spermatozoön. He says:

"Some of the spermatids stained by Henneguy's method, and nearly all of those stained by Heidenhain's method, show a spherical body near the chromatic mass (Pl. V, Figs. 232–235), and this body becomes included in the nuclear vesicle when a membrane is formed (Pl. IV, Figs. 148 and 149; Pl. V, Figs.

[1] Wilcox ('95), "Spermatogenesis of Caloptenus femur rubrum and Cicada tibicen," *Contributions from the Zoöl. Lab. of the Mus. of Comp. Zoöl. at Harvard College*, vol. xxvii, No. 1.

[2] Italics in the original.

232 and 236). I regard this body as the centrosome which is left in each spermatid after the last spermatocyte division, and I also believe it to be identical with the very conspicuous body which forms the neck of the spermatozoön (Pl. V, Figs. 196–200). The chromatic substance fuses into a smoothly contoured mass, which soon assumes a crescent shape so common in insect spermatogenesis. The neck-body lies within the nuclear membrane opposite the concavity of the chromatic crescent (Figs. 198–200). The chromatin undergoes chemical and physical changes during the metamorphosis of the spermatid, but the neck-body remains practically the same in size, and does not alter its affinity for stains. It becomes the neck of the spermatozoön (Pl. IV, Figs. 139–158; Pl. V, Figs. 196–200). The chromatic crescent is at first less dense and stains less deeply; then it becomes concentrated, and stains nearly black by Heidenhain's method. These changes in density are not well shown in the figures. At the same time it becomes elongated, one end applying itself to the neck-body, the other becoming the tip of the spermatozoön head."

Although the writer has not yet had the opportunity to examine the cells of *Caloptenus*, he cannot but regard the views of Wilcox as erroneous. The close correspondence between the body which Wilcox designates, doubtfully, a nucleolus in one place and a centrosome in another, and the structure which has, in *Xiphidium*, been traced through the various developmental stages of the spermatozoön as an accessory chromosome, indicates that the phenomena of development are quite similar in the two cases. The centrosomes in *Xiphidium*, so far as observed, are quite small, and could in no case be mistaken for such objects as Wilcox represents in the cells of *Caloptenus*.

The absence of literature has prevented any further comparative study of the subject, unfortunately, but reference must be made to the figures of Henking[1] upon the spermatogenesis of *Pyrrochoris*, in which there seems to be something similar to the appearances found in *Xiphidium*. Figs. 16 and 17 show a body marked "n" that, so far as represented, might correspond to

[1] Henking ('**91**), "Erste Entwickelungsvorgänge in den Eiern der Insecten," *Zeit. f. wiss. Zoöl.*, Bd. li, p. 685.

the accessory chromosome of *Xiphidium* in the earlier stages of its formation. Again, in Figs. 40*a*, 40*b*, 41*a*, and 41*b* appears a nuclear element, marked "x," that is clearly to be distinguished from the other chromatic structures. The same element is traced through later stages, but its ultimate fate is not indicated.

Resembling the retarded separation of the accessory chromosome in the cells of *Xiphidium*, shown in Figs. 5 and 6, is that of a pair of chromosomes shown by Henking in his Figs. 55–58. These appearances certainly indicate a resemblance between insect seminal cells that is worthy of attention.

The figures accompanying this paper are diagrammatic, and are intended merely to show the one nuclear element in the various stages of its transformations. More detailed drawings will accompany a subsequent paper, in which will be recorded a general history of the male reproductive cells of *Xiphidium*.

TECHNICAL DETAILS.

The material employed in the investigation was collected in Chicago during the months of July and August. Nymphs having the wings but scarcely developed exhibited the most complete series of reproductive cells. To fix the tissues, Flemming's fluid, Hermann's fluid, corrosive-acetic mixture, platinic chloride solution, and chromic acid combined with formalin were employed. Osmic acid mixtures gave the best results, and were finally used to the exclusion of all other fixing agents.

Sections cut $2\frac{1}{2}\,\mu$ and $5\,\mu$ thick were fastened to the slide by the water method and stained in various combinations of colors. The most satisfactory preparations resulted from double staining by means of the iron-haematoxylin method of Heidenhain, followed by eosin for a plasmatic stain. Gentian violet and eosin also produced satisfactory images. Crushed, cover glass preparations, stained like the sections, proved valuable in the determination of details where the sections were not satisfactory.

Grateful acknowledgment is hereby made to Prof. W. M. Wheeler for suggesting the line of investigation and for valuable assistance rendered in the prosecution of the same. The

work was carried on in the Hull Zoölogical Laboratory of the University of Chicago during the summer of 1898.

THE UNIVERSITY OF KANSAS,
LAWRENCE, KAN., December 1, 1898.

After the preceding had gone to press, a copy of Montgomery's paper[1] upon the spermatogenesis of *Pentatoma* was, by the kindness of Dr. Wheeler, placed at the disposal of the writer. In it is found a strong support of the views expressed in this paper upon the general character of the accessory chromosome — a support which strongly confirms the belief that this structure will be found very widely distributed, at least among the insects. Without doubt, however, specific variations of some magnitude will be found; the observations on *Pentatoma* and *Xiphidium* indicates this clearly. Much is therefore to be learned by a comparative study of different forms, and in this, objects such as *Xiphidium*, in which the element occurs with much prominence, will prove most valuable.

The results attained by Montgomery, however, are much in advance of any others up to the present time, and agree, in the main, with the appearances noted by the writer in the cells of *Xiphidium*. These are (1) the resemblance to a nucleolus in the resting stage, and (2) the similarity to a chromosome during the period of division. Montgomery expresses this briefly in the following language: "This peculiar structure acted like a nucleolus in the rest stage, but in the monaster is destined to lie in the equator among the chromosomes, where it also becomes divided in metakinesis, and so terminates by acting like a chromosome, as at the commencement it had been formed from one."

There can be no reasonable doubt of the accuracy of these observations when two investigators, working entirely independently of each other on different objects, reach the same conclusions.

[1] Montgomery ('98), The Spermatogenesis in Pentatoma up to the Formation of the Spermatid, *Zoologische Jahrbücher*, Abtheil. f. Anat. u. Ontog. der Thiere, Bd. xii.

While there exist these main points of agreement between the observations on *Pentatoma* and *Xiphidium*, minor differences may be noted. Thus there seems to be no reason to suppose that the accessory chromosome of *Xiphidium* arises by the direct transformation of one of the ordinary ones, although such a change may be possible. This does not argue against the chromatic origin of the body, however, for it is almost certainly modified chromatin, but in *Xiphidium* it arises during the resting stage and may represent derivative substance from one or all the chromosomes.

Again, its relative importance is much greater and its behavior more marked in *Xiphidium* than in *Pentatoma*. The constancy of form and structure appears to be less pronounced in Montgomery's object. As far as can be told, the staining reaction is essentially the same in both objects, and shows the same constancy that Wilcox noted for his "nucleolus." The final disposition of the body is a question that has not been decided in any case, and is one of great importance. It will, perhaps, require a knowledge of the steps in fertilization to decide positively the true character of the accessory chromosome.

Regarding the name to be applied to this structure, it would seem much more reasonable to class it with the chromosomes than with the nucleoli. The indefinite character of the latter group of bodies makes it desirable to avoid confusing the nomenclature by the addition of any more varieties to those already existing. But more important than this is the fact that during the time that chromosomes exist as such in the cell, the accessory chromosome is practically indistinguishable from the others in its behavior. It is a unit, a chromatic unit, constant in character and nearly typical in origin, transformation, and final disposition, as is believed, and corresponds well to the definition of a chromosome given by Montgomery, *i.e.*: "A chromosome is each separate chromatin element (chromatin microsomes imbedded in, or sheathed by, linin) formed in the prophases of mitosis by transverse segmentation of the spireme thread, or which, in those cases where a continuous spireme is not formed, segregates as a separate element from the chro-

matin reticulum of the resting cell; the halving of each chromosome in metakinesis results in the formation of two daughter-elements, each of which has the value of a chromosome only in the daughter-cell in which it comes to lie; that is to say, metakinesis doubles the number of chromosomes. . . . The chromosome must be ascribed an actual value (in relation to the cell generation in which it occurs) irrespective of any prospective or retrospective value."

The body under consideration fulfills the conditions of the definition, and therefore should be classed with the chromosomes. It is to be hoped that we shall soon be in a position to give it a more exact status among these chromatin elements.